TOPOGRAPHICAL RELATIONS BETWEEN ELEMENTS OF CONTROL SYSTEMS IN MAIZE

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In *Year Book 60*, parallels were drawn between gene-control systems in maize and those in bacteria. In both organisms, the comparable systems are composed, basically, of two genetic elements: an "operator" element, located adjacent to the structural gene(s) and directly controlling genic activity; and a "regulator" element that in turn controls the functioning of the operator element. Each operator responds only to a specific regulator. Therefore, each operator with its corresponding regulator represents a gene-control system. In bacteria, the position of the regulator element on the chromosome is not the same in all examined systems: it may be located either near by or at a distance from the

operator. Probably the elements of a control system in maize express similar topographical relationships; evidence supporting that probability will be presented here.

In maize, the controlling elements of a system are transposable. Therefore, different genes may come under the control of the same system, or the same gene under the control of different systems. Inception of control of gene action by a particular system sometimes occurs when the operator of the system is inserted near the locus of the gene, the regulator element being located elsewhere in the chromosome complement. At other times, inception of control is associated with the appearance of the regulator near the locus of the gene. In each such example that has been examined adequately, however, a clearly expressed two-element system has subsequently arisen, the operator element residing near the gene locus and responding to the regulator element, now located elsewhere in the chromosome complement.

With advancing knowledge of control systems in bacteria and of the topographical relations among individual components of a system, the cases in maize in which the regulator element of a known system initially occupies a position close to the structural gene require close scrutiny. In maize genetics, the devised test methods allow ready detection of the presence of the regulator element of a system, either when it is located close to the gene under the control of the system or when it is located elsewhere. When the regulator is close to the gene locus, the concomitant presence of the operator element is not so readily detected. Nevertheless, as was stated above, a clearly expressed two-element system of control of gene action does subsequently appear. The origin of such a system poses several critical questions: Are both elements of the system initially located close to the structural gene, and does the two-element system arise by removal of the regulator only? Or can a regulator element alone directly modify the action of a gene when located close to it, and does the two-element system that subsequently appears result from an alteration of the regulator element which converts it into an element that thereafter behaves as an operator?

Experiments eliciting direct answers to the above questions may not be conducted readily with maize. Nevertheless, certain relevant facts are known. Although it is possible to consider that insertion of a known regulator element close to a structural gene may initiate control of action of the gene, and also that an operator element may originate by some modification of the regulator, no certain evidence has yet been obtained of the reverse process, that is, conversion of an operator into a regulator. Moreover, examination of the behavior of each of the elements of a two-element system has shown that transposition of both elements to new locations in the chromosome complement may occur coincidently in a single nucleus, so that the operator is removed from the locus of the gene and the regulator is moved to a new location in the chromosome complement. In other cells, on the other hand, only one of the elements is transposed at a given time.

Now, if it is assumed that both an operator and a regulator are located adjacent to the structural gene in those instances where only the presence of the regulator at that location can be determined with certainty, the following possibilities are predictable. The operator and regulator might undergo simultaneous transposition, leaving the structural gene with neither element adjacent to it. Other transpositional events might remove only one of the elements, the complementary element of the system remaining in location. With regard to control of gene action, the effects produced by these kinds of transposition would lead to three distinctly different consequences. Removal of both elements would release the structural gene from control by the system to which the

elements belonged, and genetic tests would reveal the absence of the regulator element from the vicinity of the gene. Removal of the operator element only would likewise release the structural gene from control by the system to which it belonged; but the regulator element would still be located close to the gene locus. Removal of the regulator alone, however, would give rise to a clearly expressed two-element system of control of gene action—the same system that was operating before the transposition. Moreover, the location of the regulator element at some distance from the structural gene, or in another chromosome of the complement, could readily be determined.

The Cold Spring Harbor cultures include five identified instances in which the initial location of the regulator element of a known control system close to a structural gene resulted in control of the gene's action by the system to which the regulator belonged. In the three instances that have been adequately examined so far, all three of the expected consequences of transpositions outlined above have been confirmed. The evidence is reviewed below.

Three of the five examples involve the *Ac* (Activator) system, and two the *Spm* (Suppressor-mutator) system. Insertion of *Ac*, the regulator element of the *Ac* system, close to the locus of the bronze (*Bz*) gene in chromosome 9 initiated control of action of this gene by the *Ac* system. The modified locus was designated bz^{m-2}, and some discussion of it appears in *Year Books 54* and *55*. Two independently occurring insertions of *Ac* close to the locus of the *Wx* (waxy) gene in chromosome 9 resulted in control of action of this gene by the *Ac* system. These modified loci were designated wx^{m-7} and wx^{m-9}. Insertions of *Spm*, the regulator element of the *Spm* system, near the locus of the A_1 (anthocyanin) gene in chromosome 3 gave rise to two modifications designated $a_1{}^{m-2}$ and $a_1{}^{m-5}$. Extensive examinations have been made only of bz^{m-2}, $a_1{}^{m-2}$, and $a_1{}^{m-5}$.

Origin from $a_1{}^{m-5}$ of a Two-Element Control System

The original isolate of $a_1{}^{m-5}$ had an *Spm* element located close to the A_1 gene; the degree of closeness was not made apparent in the initial tests of 28 plants carrying $a_1{}^{m-5}$. In 18 of these plants, more than two *Spm* elements were present; 6 had two *Spm* elements, one obviously linked with $a_1{}^{m-5}$; and 4 had one *Spm* element, linked with $a_1{}^{m-5}$. The location of *Spm* in the immediate vicinity of the A_1 gene was not recognized in the tests conducted with these last 4 plants, because frequent transpositions of *Spm*, occurring in some sporogenous or presporogenous cells, resulted in the production of a number of gametes in which *Spm* occupied a new location in the chromosome complement. The intimate proximity of *Spm* to the A_1 locus was made evident, however, in tests of certain progeny of these plants, in which transpositions of *Spm* occurred so late in development that few or no gametes carried a transposed *Spm* element.

With respect to A_1 gene action, transposition of *Spm* away from the locus of $a_1{}^{m-5}$ leads to one or the other of two quite different results: either release of gene action from the control of the *Spm* system, or continued control by that system. Approximately half of the transpositional events that effect release from control of the *Spm* system result in an A_1 gene capable of a high level of action. The other half of these events bring about a much lower level of A_1 gene action, or occasionally the absence of such action, in both plant and kernel.

That the *Spm* system can continue to control A_1 gene action after some transpositions of *Spm* away from the $a_1{}^{m-5}$ locus was discovered in two ways. One of these utilized selected kernels on ears of plants of the constitution $a_1{}^{m-5}$ Sh_2/a_1 sh_2 (sh_2, shrunken endosperm; a_1, standard recessive allele of A_1) that had *Spm* located close to $a_1{}^{m-5}$, produced by a cross with plants homozygous for a_1 and

sh_2 and having no *Spm*. Plants were grown from 29 Sh_2 kernels that exhibited uniform anthocyanin pigmentation in the aleurone layer, intense in some kernels and pale in others. The plants were tested for presence or absence of *Spm* and, if it was present, for the number of *Spm* elements and their relative locations in the chromosome complement. When *Spm* was absent, it was introduced by means of a cross into the endosperm nuclei of kernels on the ears produced by these plants, in order to test the expression in its presence of the A_1 gene in the Sh_2–carrying chromosome. The tests indicated that, in 28 of the 29 plants, action of the gene A_1 was no longer under the control of the *Spm* system; the same level of genic expression appeared both in the presence and in the absence of *Spm*. In some of these 28 plants, no *Spm* was present. In others, one or more *Spm* elements were present but were not located close to the A_1 gene. In 2 plants, however, *Spm* was found to be located very close to the gene, even though genic action had been released from the control of the *Spm* system.

The remaining plant of the 29 was derived from a pale-pigmented kernel. Tests for the presence of *Spm* were negative. Nevertheless, the action of the A_1 gene in the Sh_2–carrying chromosome remained under the control of the *Spm* system, as was shown when *Spm* was introduced by a cross of this plant with one carrying *Spm*. The response of the gene to *Spm* was similar to that given by the class II states of a_2^{m-1} described in *Year Book 57*. With this state of a_1^{m-5}, a medium level of A_1 gene action is expressed in plants and kernels that have no active *Spm* in their nuclei, but gene action is suppressed if an active *Spm* is present. Here, then, the *Spm* system continued to control the action of the A_1 gene although *Spm* no longer occupied a position close to it. A typical two-element system of control of gene action had evolved from an apparently one-element system.

The second demonstration that not all transpositions of *Spm* away from the locus of a_1^{m-5} release the genic action from the control of the *Spm* system was provided by an examination of plants derived from other selected Sh_2 kernels from ears produced by the same type of cross as that producing the 29 kernels whose constitutions are described above. Each of these kernels exhibited a markedly altered pattern of pigmented and nonpigmented areas, as compared with that of kernels carrying the original state of a_1^{m-5}. It was suspected that each of these kernels had received an a_1^{m-5} locus whose state had been altered in a cell of the a_1^{m-5}–carrying parent plant. To test this assumption, plants derived from 10 such kernels, each selected from a different ear, were examined, and extensive tests were subsequently conducted with the progeny of 4 of them. All 10 plants carried a modified state of a_1^{m-5}, and also an *Spm* element. In 3 of the plants, the single *Spm* had remained in intimate association with the a_1^{m-5} locus; that is, the event responsible for the alteration of state had not resulted in its removal to a more distant location. In the other 7 plants, however, *Spm* was located elsewhere in the chromosome complement, and in 6 of them it was not linked with a_1^{m-5}; in the seventh, it was located approximately 30 crossover units from a_1^{m-5}. Thus a typical two-element system of control of gene action was operating in each of these 7 plants, and *Spm* was its regulator.

The illustrations given above show that with a_1^{m-5} the three anticipated consequences of different types of transposition of elements of the control system were observed: (1) Release of A_1 gene action from control by the *Spm* system, associated with removal of *Spm* from the immediate vicinity of the gene. (2) Release of such control, not accompanied by transposition of *Spm*. (3) Continued control of A_1 gene action by the *Spm* system, after removal of the *Spm* element from the immediate vicinity of a_1^{m-5}.

Analysis of a_1^{m-2}

The analysis of a_1^{m-5}, just described, proceeded rapidly as soon as plants had been isolated that carried a single *Spm* element located close to the A_1 gene. The types of gene action produced by the stable mutations were not difficult to interpret; they appeared to express different levels of standard A_1 gene action. The behavior of the modified states was also readily interpretable. Those that were associated with the two-element system of control of gene action whose origins are described above followed the same rules that had previously been established for the *Spm* system.

Analysis of a_1^{m-2}, on the other hand, has been complicated. Although the original state is similar to the original state of a_1^{m-5}, in that *Spm* resides close to the A_1 locus and the *Spm* system controls A_1 gene action, the types of expression resulting when the gene is released from the control of the *Spm* system are distinctly different. There are two classes of mutants. The first has a phenotype resembling that produced by the standard A_1 gene. The other class is composed of a series of alleles, distinguished from the first class by the distribution and intensity of pigment in plant and kernel. In the kernel, the intensity of pigmentation in the aleurone layer is not uniform, so that kernels appear somewhat mottled. The different alleles in this class may be distinguished from one another by the degree of intensity of kernel pigmentation, which ranges from very faint to fairly dark. The plants also are pigmented, but the color develops slowly and is markedly affected by sunlight: the parts of a plant exposed to direct sunlight become intensely pigmented, whereas parts not so exposed remain light in color. Although some pigment develops in the mid-rib of the leaf and at its edge, very little or none develops in the leaf blade. The two classes of mutants are thus readily distinguished. The first will be referred to as "A_1" mutants and the second as "mottled" mutants.

Control of gene action at a_1^{m-2} by the *Spm* system is quite different from the control exercised by that system when the *Spm* element is not located near the controlled gene. For example, in the modified loci a_1^{m-1} and a_2^{m-1}, in the above-described derivatives of a_1^{m-5}, and in wx^{m-8}, gene action is suppressed by an active *Spm* element but is expressed in its absence or when it is present but inactive. With a_1^{m-2} (original state), however, the reverse is true: when *Spm* is inactive, the action of the gene is suppressed; when it is active, gene action is expressed. This fact could be determined because it was possible to select some a_1^{m-2}–carrying plants in which *Spm* was in an inactive phase of long duration, and others in which *Spm* was in an active phase of long duration. Some tests conducted with plants having *Spm* in an active phase of long duration will be considered first.

Location of Spm before and after release of control of gene action at a_1^{m-2} *by the Spm system.* With the original state of a_1^{m-2}, the location of *Spm* close to the A_1 gene was established by several types of test, commencing with a cross of plants of the constitution a_1^{m-2} Sh_2/a_1 sh_2 by plants that had no *Spm* and were homozygous for a specially selected state of a_1^{m-1} and also for sh_2. A_1 gene action in these last-named plants was under the control of the *Spm* system, involving an operator element located close to the A_1 gene and an *Spm* element located elsewhere in the chromosome complement. With this selected state of a_1^{m-1}, gene action is expressed in the absence of *Spm* (or when it is present in an inactive phase). Anthocyanin pigment appears in both plant and kernel. In the kernel, pigment of medium intensity is uniformly distributed over the aleurone layer. When an active *Spm* is present somewhere in the chromosome complement, gene action is suppressed until there occurs, in some cells, a response of the operator to *Spm*

that effects a release of gene action from the control of the *Spm* system. These releases occur in a relatively few cells late in the development of plant and kernel, and most of them lead to an expression of A_1 gene action resembling that of the standard A_1 gene. Consequently, they give rise to a distinctive pattern of deeply pigmented dots in the kernel and small pigmented streaks in the plant, both appearing on a nonpigmented background. In plants and kernels carrying the original state of a_1^{m-2}, on the other hand, release of control of gene action by the *Spm* system may occur in many cells, both early and late in development. As is described above, such release may result either in a high level of A_1 gene action, a lower level that produces the "mottled" phenotype, or, rarely, a null expression of the gene. Thus, kernels carrying the original state of a_1^{m-2} and a fully active *Spm* exhibit both large and small pigmented areas of various intensities.

In the above-described cross, nearly all the Sh_2 kernels on an ear receive from the heterozygous parent either unmodified a_1^{m-2} or a modified derivative of it, and nearly all the sh_2 kernels receive the standard a_1 allele. This happens because crossing over between the locus of a_1^{m-2} and that of Sh_2 is very infrequent, not exceeding 0.12 per cent. The presence or absence of active *Spm* can be detected readily in the sh_2 kernels on the ears produced by the cross, and also in Sh_2 kernels that have received a gamete carrying a stable mottled mutant of a_1^{m-2}. If an active *Spm* is present in one such kernel, the distinctive pattern of deeply pigmented dots produced by the response of a_1^{m-1} to *Spm* appears in a mottled background. If *Spm* is absent, these dots are absent and the kernels exhibit only the mottled phenotype. Table 2 lists the phenotypes of kernels that appeared on some ears produced by the cross. The ratios of kernel types were not the same on all these ears. Nevertheless, except on ears of plants whose numbers are printed in italics, there was a direct relationship between the percentage of kernels in the Sh_2 class that received a germinal mutant of a_1^{m-2} and the percentage of kernels in the sh_2 class that received *Spm*. On ears in which all the Sh_2 kernels had received unmodified a_1^{m-2} there were no kernels in the sh_2 class that carried *Spm*. Among the ears bearing kernels that expressed germinal mutations of a_1^{m-2} the percentage of such kernels and the percentage of sh_2 kernels with *Spm* were directly related. (This correlation was exhibited among the kernels on ears having no detectable sectors derived from cells in which a stable mutation of a_1^{m-2} had occurred early in development. Ears with such sectors are not included in the table.) The correlation suggested that *Spm* was located very close to a_1^{m-2} in the heterozygous parents and that its removal from this location was associated with the origin of many of the stable mutations.

This possibility was also suggested by the phenotypes of kernels on ears produced by testcrosses conducted with other plants having the constitution a_1^{m-2} Sh_2/ a_1^{m-1} sh_2. Ears of 33 plants of this constitution were utilized in crosses with plants homozygous for a_1^{m-1} and sh_2 and having no *Spm*, and also with plants homozygous for a_1 and sh_2 and having no *Spm*. Table 3 shows the phenotypes of kernels that appeared on ears produced by the second cross. Again, a direct relationship will be noted between the percentage of kernels that received a germinal mutant of a_1^{m-2} and the percentage of kernels in the sh_2 class that received *Spm*.

The cross that produced the kernels entered in table 2 was conducted after the above-described correlation had been recognized. The ear-bearing parent plants in cultures 7979A and B, 7980A, and 7981A were derived from variegated, Sh_2 kernels on ears of plants 7799B–1, 7799B–6, and 7800A–5 of table 3. Plants were grown from kernels in the underlined classes in table 3 in order to test the conclusion that *Spm* resides close to unmodified a_1^{m-2} and that many of the

TABLE 2. Phenotypes of Kernels on Ears of Plants of the Constitution a_1^{m-2} Sh_2/a_1 sh_2 Produced by a Cross with Plants That Were Homozygous for a_1^{m-1} and sh_2 and Had No *Spm*

Plant Number	Phenotypes of Kernels							
	Sh_2 Class*					sh_2 Class†		
	Germinal Mutations			Variegated for A_1 and Mottled Spots	Percentage Germinal Mutations	Pale (No *Spm*)	Dots of A_1 in Colorless Background (*Spm*)	Percentage with *Spm*
		Mottled						
	A_1	No A_1 Dots (No *Spm*)	A_1 Dots (*Spm*)					
7979A–7	0	0	0	57	0	73	0	0
A–8	0	0	0	233	0	189	0	0
B–1	0	0	0	239	0	219	0	0
A–6	0	3	1	69	5.4	59	1	1.6
A–3	1	4	1	71	7.7	64	2	3.0
A–12	3	13	11	98	21.6	114	12	9.5
A–1	4	25	15	105	29.5	118	20	14.5
B–4	4	36	13	109	32.7	148	29	16.3
A–2	3	33	25	113	35.0	148	25	14.4
A–13	3	35	22	106	36.1	142	17	10.6
A–10	9	46	47	169	37.6	232	51	18.0
A–11	7	61	57	115	52.0	171	53	23.6
A–9	10	26	62	134	42.2	97	157	61.8
B–8	25	44	82	83	64.5	96	137	58.8
7980A–9	11	32	22	149	30.3	177	31	14.9
A–7	1	5	5	24	31.4	22	6	21.4
A–3	5	20	6	53	36.9	76	13	14.6
A–4	6	38	21	110	37.1	163	30	15.5
A–1	6	62	41	169	39.2	263	64	19.5
A–2	10	55	43	110	49.5	190	49	20.5
A–8	12	33	80	111	52.9	121	129	51.6
7981A–1	3	49	24	151	33.4	183	22	10.7
A–4	5	51	33	114	43.8	108	41	27.5
A–5	10	64	33	136	44.0	199	37	16.1
A–7	6	41	66	68	62.4	157	53	25.2
A–8	9	63	66	59	70.0	144	59	29.0

* In addition there was one pale, Sh_2 kernel.
† In addition there were three sh_2 kernels that received a_1^{m-2}.

germinal mutations arise when *Spm* is transposed to a new location in the chromosome complement. Among the 26 plants listed in table 2, 23 had one *Spm* element in the cells that gave rise to the testcross ear and 3 (whose numbers appear in italics) had two *Spm* elements. There were 4 additional plants, each also derived from an Sh_2 kernel whose endosperm was variegated. A mottled phenotype was expressed in these 4 plants rather than the phenotype produced in plants that commence development with unmodified a_1^{m-2}. The presence of a mottled mutant in these plants was confirmed by the kernel types that appeared on a testcross ear of each (rows 1–4, table 4). Since the endosperm of the kernel from which each of these plants arose started development with unaltered a_1^{m-2}, the event that produced the mottled mutant must have occurred during development of the female gametophyte in the parent plant, or in the kernel early in development of the embryo. All 4 plants had one or more active *Spm* elements. In 2 of them (7981A–3 and 7981A–6), one *Spm*, not

TABLE 3. Phenotypes of Kernels on Ears of Plants of the Constitution a_1^{m-2} Sh_2/a_1^{m-1} sh_2 That Had One Active *Spm*, Produced by a Cross with Plants Homozygous for a_1 and sh_2 and Having No *Spm*

Plant Number	Phenotypes of Kernels							
	Sh_2 Class				sh_2 Class			
	Germinal Mutations		Variegated for A_1 and Mottled Spots	Percentage Germinal Mutations	A_1	Pale (No *Spm*)	Dots of A_1 in Colorless Background (*Spm*)	Percentage with *Spm*
	A_1	Mottled						
7799B–1	0	0	201	0	0	214	1	0.46
7799B–6*	2	50	176	22.8	1	216	28	11.4
7800A–5	6	60	153	30.1	1	180	21	10.4
7984 –7	6	64	131	34.8	0	172	31	15.2
7984 –4	2	60	109	36.2	0	150	27	15.2
7984 –3	4	62	99	40.0	1	131	30	18.5
7799A	14	95	119	47.8	2	160	44	21.3

* In addition there was one colorless, sh_2 kernel on this ear. The plant derived from it had no *Spm*.

linked to the mutant locus, was present in the cells that produced the testcross ear. In the other 2, two *Spm* were present in the cells giving rise to the testcross ear—neither element linked with the mutant locus in plant 7980A–6, but one linked with it in plant 7981A–2.

The plant grown from the single sh_2 kernel containing *Spm* on the ear of plant 7799B–1 (row 1, table 3) proved to be a_1^{m-1} sh_2/a_1 sh_2 in constitution and had two independently located *Spm* elements in the cells that produced each of its tested ears.

Nine plants derived from the mottled Sh_2 class of kernels on the ear of plant 7799B–6 (row 2, table 3) were also tested for *Spm* constitution. No evidence of its

TABLE 4. Phenotypes of Kernels on Ears of Plants That Were Mottled-Mutant Sh_2/a_1 sh_2 in Constitution, Produced by a Cross with Plants Homozygous for a_1^{m-1} and sh_2 and Having No *Spm*

Plant Number	Phenotypes of Kernels			
	Mottled Sh_2 Class		sh_2 Class	
	No Dots of Deep Pigmentation (No *Spm*)	Dots of Deep Pigmentation (*Spm*)	Pale (No *Spm*)	Deep-Pigmented Dots in Colorless Background (*Spm*)
7980A–6	86	162	73	149
7981A–3	120	107	120	111
7981A–6	82	81	74	82
7981A–2	92	125	111	55
7980B–3	91	85	103	80
7980C–2	60	141	166	45
7980B–4	30	211	95	127
7981B–1	20	219	254	22
7981B–6	0	274	255	1
7981B–8	80	126	149	71
7981C–3	47	175	51	180

presence was shown by the kernels on the ears of 6 of these 9 plants; but it was present in the cells that gave rise to the testcross ear in the remaining 3 plants (7980B and C, table 4). Plant 7980B–3 had one *Spm*, not linked with Sh_2; plant 7980C–2 had one *Spm*, linked with Sh_2; and plant 7980B–4 had two *Spm*, one linked with Sh_2. Testcrosses conducted with 8 of the 12 plants derived from mottled Sh_2 kernels on the ear of plant 7800A–5 (row 3, table 3) produced no evidence of the presence of *Spm*. It was present, however, in the remaining 4 plants, as indicated in table 4. Very close linkage of *Spm* with the locus of the mottled mutant was exhibited by plant 7981B–6. Linkage of *Spm* with the locus of the mutant was expressed in plants B–1 and B–8. The ratio of kernel types on the ear of plant 7981C–3 (355 with *Spm*/98 with no *Spm*) suggests the presence of at least two *Spm* elements, not linked with Sh_2, in the cells that gave rise to this ear.

All together, 42 plants derived from mottled Sh_2 kernels on ears of a_1^{m-2} plants that had one *Spm*, located close to a_1^{m-2}, have been tested for *Spm* constitution and location. Twenty-six of the plants showed no evidence of the presence of *Spm*. Twelve plants had one *Spm*: in 2 of them it was situated very close to the locus of the mutant; in 2 others it was linked with the mutant locus; and in the remaining 8 there was no evidence of such linkage. Three plants had two *Spm*; neither was linked with the locus of the mutant in 2 of the plants but one *Spm* was linked with it in the third. The remaining plant of the 42 had three *Spm* elements, none of them linked with the mutant locus. Thus, *Spm* was present in only 16 (38 per cent) of the 42 plants derived from kernels in which a chromosome carrying a germinal mutation was received by both the endosperm and the zygote nuclei. Among the 1288 mottled Sh_2 kernels in table 2 that appeared on ears of plants having one *Spm*, 552 (43 per cent) carried *Spm* and 736 had no *Spm*. The agreement in distribution of *Spm* to the mutant class, demonstrated by these two types of test, is good.

Further confirmation that *Spm* was located close to a_1^{m-2}, and that its removal from that location was related to the origin of the stable mutants, was provided by tests of the progeny of an $a_1^{m-2}\ Sh_2/a_1\ sh_2$ plant produced by crossing this plant with one that was homozygous for a_1^{m-1} and sh_2 and had no *Spm*. A very large sector, present in the a_1^{m-2}–carrying plant, was derived from a cell in which a mutation to a stable mottled allele had occurred. On the ear of the described testcross there were 306 mottled, Sh_2 kernels, of which 150 carried *Spm* and 156 had no *Spm*. Only 18 Sh_2 kernels on this ear had received unmodified a_1^{m-2}. Among the 315 sh_2 kernels on this ear, 177 were uniformly pigmented (no *Spm*) and 138 had dots of deep pigmentation in a colorless background (*Spm* present). Ten plants grown from mottled Sh_2 kernels carrying *Spm*, and 10 plants from Sh_2 kernels that had received unmodified a_1^{m-2}, were tested for *Spm* number and location. On testcross ears produced by the 10 plants derived from the mottled kernels the ratio of kernel types indicated that 9 of them had one *Spm*, not linked with Sh_2, and that two *Spm* elements, not linked with Sh_2, were present in the cells that produced the ear on the tenth plant. On testcross ears of the 10 plants derived from kernels having unmodified a_1^{m-2}, the ratios of kernel types were similar to those entered in table 3: one *Spm* was present in each plant, and it was located close to a_1^{m-2}. It may be concluded, then, that the a_1^{m-2}–carrying parent of these 20 plants commenced development with a single *Spm*, located close to a_1^{m-2}. Early in development of that plant, transposition of *Spm* to a new location, occurring in one cell, led to the origin of the stable mottled mutant that was present in all descendants of the cell.

From the above-described series of tests, it is evident that the origin of many

of the stable mutants of a_1^{m-2} is associated with transposition of *Spm* to a new location in the chromosome complement. In some of the stable a_1^{m-2} mutants, on the other hand, *Spm* continues to occupy a position close to the locus of the modified A_1 gene. Thus, in these respects, a_1^{m-2} and a_1^{m-5} are comparable.

Origin of a two-element system of control of gene action at a_1^{m-2}. Although the tests aimed at identifying events in which a clearly expressed two-element system of control of gene action arises from a_1^{m-2} have not yet been completed, one example may have been found. A kernel with a distinctive phenotype appeared on an ear of a plant that was a_1^{m-2} Sh_2/a_1 sh_2 in constitution and had one *Spm* located close to a_1^{m-2}, after it was crossed with a plant of similar constitution. The selected kernel was weakly and irregularly pigmented, with no spots of deep pigmentation in its aleurone layer. The phenotype of the plant grown from this kernel was similar to that expressed by many of the stable mottled mutants of a_1^{m-2}, since anthocyanin pigment appeared in the same regions of the plant. Testcrosses conducted with this plant gave no evidence of the presence of *Spm*. Its constitution, however, proved to be a_1^{m-2} Sh_2/a_1 sh_2. The kernels on one of its ears were produced by a cross with a plant homozygous for a_1 and sh_2 and having no *Spm*. All the Sh_2 kernels on this ear exhibited the same phenotype as that shown by the kernel that gave rise to the plant. The kernels on a second ear of the plant were produced by a cross with a plant that was homozygous for a_1 and sh_2 and carried one *Spm* closely linked with the *Pr* marker in chromosome 5 (*Pr*, purple aleurone; *pr*, recessive allele, red aleurone). Of the 217 Sh_2 kernels on this ear, 102 were variegated for pigmented areas of different intensities, in a pattern resembling that produced by unmodified a_1^{m-2}. The phenotype of the remaining 115 Sh_2 kernels was similar to that on the first ear, just described. From the close linkage of the variegated phenotype with *Pr* and the nonvariegated phenotype with *pr*, it is concluded that gene action at this modified a_1^{m-2} locus is under the control of the *Spm* system, although *Spm* no longer resides close to the locus.

The modified a_1^{m-2} locus present in the plant just described could have arisen from removal of only the *Spm* element from the vicinity of the a_1^{m-2} locus, the operator element remaining in location. That inactivation of the *Spm* element was responsible for the modification is not probable, as it is well established that such inactivation results in suppression of the action of the A_1 gene (see below); much pigment appeared in the plant having this modified a_1^{m-2} locus, and some pigment appeared in the aleurone layer of the kernels.

Inactive Spm at the locus of a_1^{m-2}. Testcrosses conducted with plants in which *Spm* was in an inactive phase, changing to an active phase only in a few cells very late in development, also served to place *Spm* close to the locus of unmodified a_1^{m-2}. Two types of testcross were performed. When plants with inactive *Spm*, a_1^{m-2} Sh_2/a_1 sh_2 in constitution, were crossed with plants homozygous for a_1 and sh_2 and having no *Spm*, all the kernels on some ears were colorless. On other ears, however, a few kernels in the Sh_2 class had a sector containing pigment of light intensity, and some of these sectors, in turn, also displayed small dots of deep pigmentation. Occasionally, the entire aleurone layer of an Sh_2 kernel exhibited such dots on a lightly pigmented background. Also, an occasional Sh_2 kernel was variegated throughout its aleurone layer, with large as well as small pigmented areas of various intensities. Tests conducted with plants derived from such variegated kernels indicated that the phenotype of the kernels was produced by a change in phase of activity of *Spm*, from inactive to active, occurring in a cell late in the development of the a_1^{m-2}–carrying plant.

The second type of test utilized the same plants as those described above, as

ear-bearing parents in crosses with plants that were homozygous for a_1^{m-1} and sh_2 and had no *Spm*. On some of the resulting ears, all kernels in both the Sh_2 and sh_2 classes were uniformly pigmented and showed no evidence of the presence of *Spm*. On other ears, a few of the kernels in the Sh_2 class exhibited sectors of much lower pigment intensity, and some of these, in turn, had small deeply pigmented spots. Occasionally, the whole aleurone layer of an Sh_2 kernel exhibited this small-spotted phenotype, or one with large as well as small areas of different grades of pigment intensity. Kernels having these last two phenotypes would be expected to appear if, in the a_1^{m-2}–carrying plant, the inactive *Spm* underwent a change to the active phase in some cells, late during development of the ovule or in the female gametophyte.

When plants of the constitution a_1^{m-2} Sh_2/a_1 sh_2 that carried an inactive *Spm* were crossed with plants homozygous for a_1 and sh_2 that carried one or more active *Spm* elements, all or nearly all kernels that received a_1^{m-2} from the heterozygous parent and no *Spm* from the homozygous parent were colorless. In contrast, all those that received active *Spm* from the homozygous parent exhibited many mutant areas, in a pattern resembling that produced by a_1^{m-2} when the *Spm* adjacent to it is in an active phase.

Conclusions derived from the study of a_1^{m-2}. Results of the described tests with plants having unmodified a_1^{m-2}, and with others having modified derivatives of the locus, indicate that certainly two and probably all three of the predicted consequences of transpositional events, outlined early in this report, have been observed.

In this report, some aspects of the analysis of a_1^{m-2} have been considered in detail, not only in order to develop the thesis stated earlier but also to indicate the nature of the evidence that makes it possible to relate the mode of control of the *Spm* system to that controlling the alternate action of the duplicate genes, H_1 and H_2, associated with flagella antigen formation in the bacterium *Salmonella*. In maize plants that are a_1^{m-2}/a_1^{m-1} in constitution, with an *Spm* element located close to a_1^{m-2}, which of the alleles will be active and which inactive is determined by the phase of activity of the *Spm* element. In *Salmonella*, which of the two duplicate genes will be active and which inactive is determined by the phase of activity of the controlling element *Vh*, located close to the H_2 gene.

The Derivatives of bz^{m-2}

Early studies of bz^{m-2} were reported in *Year Books 54* and *55*. It was shown that *Ac*, the regulator of the *Ac* control system, resides close to the locus of the bronze gene in chromosome 9, and also that the *Ac* system controls the action of this gene. The behavior of unmodified bz^{m-2} was examined, initially, in 172 plants carrying bz^{m-2} in one chromosome 9 and the standard stable recessive, *bz*, in the homologue, as well as in 13 plants homozygous for unmodified bz^{m-2}. Subsequent studies were conducted with plants carrying modified derivatives of bz^{m-2}. Since the information obtained is both diverse and extensive, the present report will be confined to summary statements pertinent to the topic in hand.

In a cross of bz^{m-2}–carrying plants to plants homozygous for standard *bz*, kernels that had received a modified derivative of bz^{m-2} appeared on some ears. Most of these kernels exhibited either a null level or a high level of gene action at the bronze locus. It was suspected that in them the action of the bronze gene, derived from bz^{m-2}, had been released from the control of the *Ac* system. To test this conjecture, selections were made of 35 independently occurring examples of change of bz^{m-2} to an apparently stable null-expression allele, and of 14 independently occurring changes to an allele expressing a high level of gene action. It could be determined readily that, in 33 of the 35

selected examples, release of gene action from control by the *Ac* system was associated with the origin of a stable null expression of the bronze gene. In 19 of these 33, *Ac* was absent from chromosome 9 in the original plant carrying the modified bz^{m-2} locus, although it was present elsewhere in the chromosome complement in 6 of the 19 plants. In the rest of the 33 plants (14), *Ac* was present in chromosome 9. It was located close to the bronze gene in 2 of them, and at positions away from the locus in 3 others; but its exact location was not determined in the remaining 9 plants, 1 of which had two *Ac* elements, one in chromosome 9 and one elsewhere.

The 2 remaining kernels of the 35 that were selected for a stable, null expression of the bronze gene produced plants in which no *Ac* was present; both plants were totally bronze in phenotype. When they were crossed with plants carrying *Ac*, it was learned that the bronze gene, derived from bz^{m-2}, was under the control of the *Ac* system. The manner of its response to *Ac* was similar to that observed in the many other examples of two-element control systems in which *Ac* is the regulator element. In both these plants, and in a third plant derived from a kernel selected in a different manner, a two-element system of control of gene action had arisen from bz^{m-2}. Although *Ac* was no longer located close to the bronze gene, it continued to be the regulator of the system controlling its action.

The 14 original kernels selected for the presence of a derivative of bz^{m-2} that expressed a high level of gene action gave rise to 8 plants having *Ac* and 6 having no *Ac* in their nuclei. Seven of the 8 *Ac*-carrying plants had one *Ac* element. In 4 plants it was not linked with markers in the short arm of chromosome 9; in the other 3 it was linked with such markers, being located close to the *Bz*-expressing gene in 2 of them and proximal to the locus of *Wx* in the third. The eighth *Ac*-carrying plant had two *Ac*, one located close to the *Bz* gene and one not linked with markers in the short arm of chromosome 9. Tests of all 14 plants and their progenies indicated that the presence of an *Ac* element in the nucleus did not effect a modification in action of the *Bz*-expressing gene derived from bz^{m-2}. Action of this gene appeared to have been released from the control of the *Ac* system.

A modified state of bz^{m-2} was recognized early in the study of that locus. The alteration at the bronze locus that produced this state did not remove *Ac*, which remained close to the locus of the gene, and the *Ac* system continued to control gene action. In contrast to the original state of bz^{m-2}, this altered state is characterized by a high level of bronze gene action. Some of its *Ac*-controlled modifications result in recognizable changes in level of gene action. Others result in release of the gene from control by the *Ac* system, and such release is often associated with maintenance of a high level of gene action. Still other modifications give rise to further altered states. One of these resembles the initial state of bz^{m-2}, and another has proved to be instructive for the thesis of this report, for it allows ready selection of kernels produced from cells in which *Ac* no longer occupies a position close to the bronze gene. This state was recognized, initially, in a single kernel on an ear. The aleurone layer of the kernel exhibited many deeply pigmented spots in a lightly pigmented background.

Tests of the plant derived from this kernel, and of its progeny, showed that *Ac* occupied a position close to the locus of the modified bronze gene and that the observed changes in action of the gene were expressions of control by the *Ac* system. On ears produced by a cross of plants carrying this state with plants that were homozygous for the standard recessive *bz* and had no *Ac*, kernels that received the state exhibited deeply pigmented areas in a lightly pigmented background. A few kernels on some ears,

however, showed only the light background pigmentation, with no deep-colored spots. Plants derived from 5 such kernels were examined. Tests were made for the presence or absence of *Ac* in them, and for the expression of the weak bronze allele in their progeny produced by a cross with plants that were homozygous for standard *bz* and had either no *Ac* or one or more *Ac*. It was learned that no *Ac* was present in these plants. In the absence of *Ac*, the expression of the bronze gene is constant, and it behaves as a weak allele of *Bz*. When *Ac* is present, however, deeply pigmented spots appear in a lightly pigmented background. It could be determined readily that these spots arise through the response of an operator element at the locus of the weak *Bz* allele to the presence of *Ac*. Thus, a two-element system of control of gene action was expressed in each of these 5 selected examples, and *Ac* was the regulator of the system.

The above-described sequence of events affecting bz^{m-2} may be interpreted in the following manner. Insertion of the operator and regulator elements of the *Ac* system close to the locus of the standard *Bz* gene gave rise to bz^{m-2}, which exhibits a null base level of gene action. Thus, the original bz^{m-2} locus may be symbolized as *bz(op)Ac*. Since only the *Ac* element at the locus of the gene has been determined, the symbol for the operator element (*op*) is shown in parentheses. Removal of both the operator and the regulator, *Ac*, from the locus of the gene, or removal of the operator alone, releases the gene from control by the *Ac* system. Removal of *Ac* alone allows the presence of the operator to be recognized, and the locus can be given the symbol *bz-op*. A change at bz^{m-2}, assumed to be induced by the operator in response to the regulator *Ac*, produces a new state characterized by a high level of gene action, which is symbolized $Bz^{s}(op)Ac$. A subsequent modification, again assumed to be induced by the operator, gives rise to another state characterized by an intermediate level of gene action, which is symbolized

TABLE 5. Response of the Bronze Gene to *Ac* in Selected Derivatives of *bz(op)Ac*, the Original State of bz^{m-2} (Part I), and in Two of Its Modified States, $Bz^{s}(op)Ac$ (Part II) and $Bz^{w}(op)Ac$ (Part III)

Expression of Bronze Gene in Selected Kernel	Response of Bronze Gene to *Ac*	Symbol for Bronze Gene	No. Cases Examined
	Part I		
Null expression; stable	Negative	*bz*	22
	Negative	*bz–Ac*	2
	Negative	*bz*; *Ac* in chromosome 9; position not determined	9
	Positive	*bz–op*	3
High level of gene action; stable	Negative	Bz^{s}	11
	Negative	Bz^{s}-*Ac*	3
High level of gene action; unstable	Positive	$Bz^{s}(op)Ac$	1
	Part II		
High level of gene action; stable	Negative	Bz^{s}	5
	Negative	Bz^{s}-*Ac*	7
Return to bz^{m-2} expression	Positive	*bz(op)Ac*	10
Weak expression of gene; unstable	Positive	$Bz^{w}(op)Ac$	1
	Part III		
High level of gene action; stable	Negative	Bz^{s}	3
Weak expression of gene; stable	Positive	Bz^{w}-*op*	5
Return to high level of gene action; unstable	Positive	$Bz^{s}(op)Ac$	1

Bz^{w}(*op*)*Ac*. Removal of only *Ac* allows the presence of the operator to be recognized, and in this event the gene locus is given the symbol *Bz*^{w}-*op*.

Table 5 summarizes the evidence for the considerations outlined in this section. The symbols in the table are the same as those just described. A symbol that does not include *op* or *Ac* indicates that no evidence has been obtained of the presence of either element at the locus of the gene.

The findings presented in this report are sufficiently extensive to leave no doubt that a two-element system of control of gene action, composed of an operator element at the locus of the gene and a regulator element located elsewhere, may arise at a gene locus that initially carried the regulator of the system. Although in the examples studied the origin of the operator element has not been determined directly, it is nevertheless evident that the predicted consequences of removal of either or both elements from the locus of the gene, on the assumption that both were present initially, have been confirmed.